Green tea and vitamin C ameliorate some neuro-functional and biochemical signs of arsenic toxicity in rats

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Abstract

Background/objectives: Nervous system damage is one of the consequences of oral exposure to waterborne inorganic arsenic. In this work, the role of oxidative status in the neurotoxicity of arsenic, and the possible role of two foodborne antioxidants in ameliorating the arsenic-related oxidative stress, were investigated. **Methods:** Male Wistar rats were given 10 mg/kg b.w. trivalent inorganic arsenic (in form of $NaAsO_2$) 5 day per week for 6 weeks by gavage, combined with vitamin C solution (1 g/L) or green tea infusion (2.5 g in 500 mL boiled water) as antioxidants given in the drinking fluid. **Results:** Body weight gain was reduced by arsenic from the 2nd week and the antioxidants had no effect on that. Cortical evoked potentials had increased latency, and tail nerve conduction velocity was reduced, and this latter effect was counteracted by the antioxidants. The effect of green tea was stronger than that of vitamin C, and green tea also diminished lipid peroxidation induced by As. There was fair correlation between brain As levels, electrophysiological changes, and lipid peroxidation, suggesting a causal relationship. **Discussion:** Natural antioxidants might be useful in protection of the CNS against the toxicity of oral As.

Keywords

Arsenic; Neurotoxicity; Evoked electrical activity; Oxidative stress; Antioxidant; Rat

Introduction

Several antioxidants are known and/or supposed to exert health-promoting effects or to protect against toxic and other influences involving oxidative mechanism. The nervous system is prone to oxidative damage, due to highly active energy production in mitochondria, resulting in some reactive oxygen species escaping the mitochondrial reaction chain, to abundance of (unsaturated) structural lipids, and to low antioxidant defence capacity in the brain[1]. The putative neuroprotective action of antioxidants has been verified in rat models of ischemia-induced memory impairment[2] and Alzheimer's disease[3]. Also, human dopaminergic cells could be protected *in vitro* from rotenone-induced damage by the antioxidant rutin[4].

Oral exposure to inorganic arsenic (As) due to contaminated drinking water is a public health risk affecting tens of millions in different parts of the world. Arsenic as a chemical element is the 20th most abundant element in the Earth's crust and is ubiquitous in trace amounts[5]. Its presence in subsurface waters used for drinking is due to geochemical factors: arsenic had been bound in ferric oxide-hydroxide containing layers in the geological past, at the time of sediment formation, and is being released when the local redox conditions in the bedrock aquifer are changed, typically due to human interference resulting from drilling deep wells and drawing water [5]. This problem has been present in regions of Asia (Bengal Basin in India/Bangladesh: up to 3.000 μg/L) and South America (Argentina: up to 10.000 μg/L)[6]. In the Carpathian Basin, more exactly in South-East Hungary and the adjacent Romanian and Serbian regions[7], the problem has become less severe by now but municipal drinking water with As concentration higher than the European Union limit value of 10 μg/L is still found.

Neurotoxicity is an important aspect of the harmful effects of arsenic. Encephalopathy with mental alterations and coma developed in several members of a family after waterborne arsenic exposure from their private well[8]. In workers of a copper smelter who were exposed to

the arsenic content of the ore processed, alterations in EEG, visual evoked potentials, and electroneurography were found[9]. Corresponding effects in animal models of arsenic intoxication have also been described[10,11].

Generation of reactive oxygen and nitrogen species is one of the ascertained mechanisms of action for arsenic[12] and is likely involved in its effects on the nervous system [13]. In peripheral nerves of As-exposed rats, local As deposition, increased lipid peroxidation, and decreased conduction velocity were found to develop in parallel[10]. Similar relation between As exposure, oxidative stress and neurological symptoms was found in the copper smelter workers mentioned above[9]. The involvement of oxidative stress suggests that antioxidants may potentially be protective against As-induced damages of the nervous system.

For the present study, two antioxidants were chosen, vitamin C (ascorbic acid) and infusion made from green tea; both being well-known and easily accessible to the public, and also generally accepted as agents of natural (as opposed to synthetic) origin. Ascorbic acid is a chemically defined and well-described antioxidant, often used as reference substance in determining antioxidative capacity of samples[14]. Besides, it is a natural substance present in various foods and drinks, and was reported to diminish depletion of reduced glutathione and subsequent cellular damage after As exposure in rats[15]. Infusion of green tea leaves, a popular drink worldwide, contains numerous antioxidants, first of all catechins. Its neuroprotective effects, based on antioxidant (and chelating) activity have also been reported[16]. Our aim was to test whether the mentioned two antioxidants can diminish general toxicity and nervous system functional damage – detected by electrophysiological and biochemical methods – in rats exposed orally to inorganic arsenic.

Materials and Methods

Animals and treatment

The experiment was carried out on young adult Wistar rats, 6 weeks old at start with ca. 200 g body weight. The rats were purchased from Toxi-Coop (Hungary), and were housed, with three or four rats in one cage, in a GLP-rated animal house (22±1ºC, 40-60% relative humidity, 12-h light/dark cycle with light on at 06:00), and had free access to standard rodent chow and to the drinking fluid which was either plain tapwater (of low arsenic content, 7 μg/L, as stated by the municipal waterworks) or an antioxidant solution described below.

There were 6 groups of rats with 10 animals each at start, and the treatment period lasted 6 weeks. All rats had a combined treatment with substances given by gavage and via the drinking fluid. Control rats (group *Con*, see Table 1) received plain tapwater for drinking and distilled water by gavage. Arsenic-treated rats (groups *As, AsC and AsT*) received $NaAsO_2$ by gavage (8.67mg/mL $NaAsO_2$ dissolved in distilled water) 5 days per week (see Table 1 for groups and doses). In groups *AsC* and *AsT*, arsenic treatment was combined with administration of the antioxidants vitamin C and green tea infusion, respectively, via the drinking fluid; while the rats in group *VitC* and *Tea*, received one of the two antioxidants alone and distilled water by gavage. The antioxidant-containing drinking fluid was given freshly each Monday, Wednesday and Friday morning and removed 48 hrs later (measurements showed that antioxidant activity was not lost during this period). This way, the rats were exposed to the antioxidants for 6 days a week. The volume consumed by the rats was measured in order to follow antioxidant intake.

It was supposed that the oral-only mode of administration chosen for the present study gives a realistic model of drinking water-borne As exposure and antioxidant uptake by food and drinks. Dose and chemical form of As in this work was based on[17]. The dose and way of

application of green tea was similar to several literary sources[18,19] and its effect on certain CNS functions affected by As in rats has been observed[17]. Vitamin C was chosen as a standard antioxidant, the dose being set so that effective antioxidant action (measured by FRAP method, see below) be in the same range with that of green tea. $NaAsO_2$ was purchased from Sigma-Aldrich (Hungary) while the two antioxidants were obtained at a local pharmacy shop.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed. The methods used in the experiments were licensed by the authority competent in animal welfare issues under No. XXI./151/2013.

General toxicology

During the treatment period, the rats' body weight and general health state was recorded daily. Body weight data were used to determine the daily dose for each rat and to graphically demonstrate the effects on weight gain.

After electrophysiological recording at the end of the experiment (see below) the rats were overdosed with urethane and were dissected. From the groups *Con*, *As*, *AsC* and *AsT*, three rats per group were randomly chosen. These were transcardially perfused with 500 ml PBS to remove blood from the organs, and their whole brain - as the primary target of this study - as well as liver, kidneys, and 2-3 ml of red blood cells (RBCs, separated by centrifugation from heparinised whole blood drawn before perfusion) - to see the parallel systemic effects - were shock-frozen in liquid nitrogen, and stored at -20°C for As level determination and biochemical measurements.

Electrophysiological investigation

One day after the last treatment, the rats were anaesthetised by ip. injection of urethane (1000 mg/kg b.w.[21]; and the left hemisphere was exposed as described in[11]. For recording evoked cortical activity (evoked potentials, EPs), ball-tipped silver recording electrodes were placed on the dura over the primary somatosensory (SS) projection area of the whiskery pad (barrel field), and over the primary visual (VIS) and auditory (AUD) foci; located on the basis of a somatotopic map[22] and own previous experience. A stainless steel clamp, attached to the cut skin edge, was used as indifferent electrode.

The SS EP was elicited by square electric pulses (3-4 V; 0.05 ms) delivered via a pair of needles inserted into the contralateral whiskery skin. VIS stimulation was performed by flashes delivered by a high-luminescence white LED directly into the contralateral eye of the rat. For AUD stimulation, clicks (ca. 40 dB) were applied into the contralateral ear of the rat from a mini earphone through the hollow ear bar of the stereotaxic frame. Fifty stimuli of each modality per rat were applied. VIS and AUD stimuli were given with 1 Hz frequency and SS stimuli, with 1, 2 and 10 Hz. It was supposed that by varying the frequency of SS stimulation, the dynamic interaction of successive excitation processes in the sensory system can be assessed, which in turn reflects the actual state of the CNS[23]. The 50 EPs were averaged, and onset latency was measured between time zero (stimulus artefact in case of SS stimulation) and the start of the main wave of the EP as exemplified for SS EPs in Fig. 2B.

The state of the peripheral nerves was tested by eliciting compound action potentials (CAPs) in the tail nerve. A pair of stimulating electrodes (delivering 4-5 V, 0.05 ms square pulses at 1, 20, 50 and 100 Hz frequency) was inserted at the base of tail, and a pair of recording electrodes, 50 mm distally. Conduction velocity was calculated from the latency of the CAP at the recording electrodes and the 50 mm distance. The complete

electrophysiological recording and evaluation was performed by the software NEUROSYS 1.11 (Experimetria Ltd., Hungary).

Chemical and biochemical measurements

To determine As concentration, samples of RBCs, liver and kidneys (ca. 1 g each) and cerebral cortex (ca. 0.2 g, detached from the frozen whole brain) were dried at 80°C to constant weight, and were digested in 4 mL (cortex: 0.8 mL) 65% HNO_3 at 90°C for 90 min. The digested matter was diluted 25-fold and As determination was done by inductively coupled plasma mass spectrometry at the Department of Inorganic and Analytical Chemistry, University of Szeged Faculty of Science and Informatics.

From the cerebral cortex and RBC samples (of the same animals used also for the As measurement) indicators of oxidative stress were determined by the co-authors from the Department of Biochemistry and Molecular Biology, University of Szeged Faculty of Science and Informatics. Protein content of the samples (for calculation basis) was measured – after haemolysing of the RBCs, homogenizing the cortex samples, and diluting them as appropriate – by the method of Lowry[24]. The primary oxidative insult caused by As exposure was assessed by measuring the reactive oxygen species hydrogen peroxide (H_2O_2), based on oxidation of o-dianizidine by horse radish peroxidase using H_2O_2 in the reaction mixture[25]; and reactive nitrogen species peroxynitrite anion (ONO_2^-), based on its differential breakdown at neutral vs. alkaline pH[26]. Both oxidative molecules have been described to be involved in the damaging effect of As[13]. The biomolecular damage was characterized by measuring lipid peroxidation using the thiobarbiturate reaction[27] against malonic dialdehyde standard.

The antioxidant capacity of vitamin C solution and green tea infusion was determined at the Department of Food Engineering, University of Szeged Faculty of Engineering by the FRAP method[28] in ascorbic acid equivalents, in samples taken from the watering bottles in freshly prepared state and after they were used in the cages for 48 hours. Total phenol content of the green tea infusion was determined by the Folin-Ciocalteau method[29].

Data analysis

The distribution of data was checked for normality by means of the Kolmogorov-Smirnov test. Data analysis was done by one-way ANOVA. Post hoc analysis of group differences was performed by Scheffe's test, setting the probability level at $p<0.05$. Linear correlation between data sets was checked by the "linear fit" function of MS Excel.

Results

General toxicity

Calculated from the doses given in Table 1 and from the measured daily body weights, the summed amount of As received by the rats was calculated (Table 2). This amount of arsenic caused considerable reduction of body weight gain which was visible already after one week (i.e., after only 5 doses of As) and was significant from the 3rd treatment week on (Fig. 1). The overall weight gain during the 6 weeks of treatment was in each group receiving As significantly lower than in the corresponding As-free group (Table 3). So, according to the data, neither vitamin C nor green tea could diminish the effect of As on body weight.

Electrophysiological changes

The most characteristic effect of As on the cortical EPs was lengthening of the latency. On the SS EP (Fig. 2A) latency increase on As treatment was significant at each stimulation frequency vs. *Con;* and the frequency dependence (extra lengthening vs. 1 Hz stimulation) was also more pronounced in group *As*. In group *AsC*, the effect was partially reversed while in *AsT* the latency was ca. equal to that in *Con*. In case of VIS and AUD EPs (Fig. 2C) the effects were identical: lengthened latency in *As*, the effect of As antagonized partly in *AsC* an almost fully in *AsT*.

The CAPs recorded from the tail nerve indicated decreased conduction velocity in *As* vs. *Con*, the effect being reversed more by green tea than by vitamin C. The frequency dependence of the conduction velocity was, however, not much altered by As and/or the antioxidants (Fig. 3).

Arsenic levels and oxidative stress indicators

Arsenic treatment caused a massive increase of the As content in the treated rats' organ samples (4-6 fold in the cortex, RBCs and liver, and ca. 40-fold in the kidneys) compared to the background value found in the untreated controls (*Con* in Table 2). The only noteworthy effect of the antioxidants on tissue As levels was the substantial (although not significant) reduction in the liver by green tea (*AsT* vs. *As*).

The level of H_2O_2 and ONO_2^- as reactive oxygen and nitrogen species was minimally increased in the As-treated rats' cortex samples and the effect of antioxidants on that was also slight. In the RBCs, As caused a more marked increase (significant in case of peroxynitrite), and the counter effect of the antioxidants was also visible (Table 4). The intensity of lipid

peroxidation, however, indicated by the level of TBARS, increased significantly both in the RBCs and in the cortex, and was significantly diminished in the cortex by green tea but not by vitamin C.

The measured antioxidant power of vitamin C solution was finally somewhat higher than that of the green tea infusion (in ascorbic acid equivalents – vitamin C: fresh, 0.70±0.12 mg/ml; 48 hrs, 0.38±0.06 mg/ml; green tea: fresh, 0.39±0.09 mg/ml; 48 hrs, 0.29±0.13 mg/ml). However, as green tea was more efficient in counteracting the neuro-functional and biochemical effects of As (shown by Fig 2 and 3, and Table 4) it could be supposed that properties of the drinking fluids other than antioxidant capacity, such as the high total phenol content of 0.90 ±0.11 mg/ml in the fresh infusion, also had an effect on the outcome.

Correlation between... REMOVED

Discussion

Oral As exposure by gavage, as applied in the present experiment, resulted in massive, significant deposition of As in the treated rats' blood and organs (Table 2), and the As levels measured in our work were in the same order of magnitude with those of comparable experiments. After 10 mg/kg b.w As given for 4 weeks by gavage, ca. 10 mg/kg As were measured in the brain[30]; and in another study, ca. 1.2 mg/kg As after only 2 mg/kg b.w. for 4 weeks[31]. In a peripheral nerve, nearly 200 mg/kg As were found after 10 mg/kg b.w. given daily for 30 days[10]. These reports made it more likely that the alterations observed in our work were in fact caused by As.

There are numerous reports on CNS damage by As in humans (reviewed in[32]) but electrophysiological data are presented only in a few of them. Complete loss of visual, and

reduced amplitude of auditory, EPs were seen in a patient who consumed 11 g of $NaAsO_2$ in a suicide attempt[33]. Long-lasting reduction of nerve conduction velocity and abnormal action potentials were detected in other patients surviving a single dose of As[34]. In the As-exposed copper smelter workers, lengthened latency of the pattern reversal visual EPs, together with various peripheral nerve abnormalities, were observed more frequently than in non-exposed control employees of the same factory; and evidence for causal relationship of the functional damages with elevated As load and to oxidative stress were found[9]. Simultaneously with the highly elevated As level in the sural nerve of As-exposed rats, slowed conduction velocity and increased lipid peroxidation were developing[10]. It has been generally accepted by now that oxidative effect is a major element of toxic mechanisms of As[13].

Our results, indicating slower reaction on a stimulus in the central sensory systems (Fig. 2) and in the tail nerve (Fig. 3) on As exposure, were in line with these findings. Moreover, the reversal of the effect of As by the antioxidants, shown in Fig. 2 and 3, suggested that oxidative damage was involved in the neuro-functional changes observed in the As-treated rats in our study.

Generally, both the peripheral and the central parts of the nervous system are prone to oxidative damage, due to highly active mitochondrial energy production, to abundance of (unsaturated) structural lipids, and to low antioxidant defence capacity in the brain[1]. Reactive oxygen species may constitute the final common pathway of effect of several neurotoxicants[35] and oxidative damage to membrane lipids in axons and neuronal cell bodies may lead to changes of fluidity and probably to alterations of membrane-bound functions crucial to the functioning of neurons[36]. Synaptic transmission and regenerative nerve pulse conduction both depend clearly on normal membrane functioning which explains why the pattern of changes of EP latency and tail nerve conduction velocity were similar.

On the other hand, it is also becoming more and more accepted that antioxidants may be of use in the prevention and therapy of brain damage and/or dysfunction; including Parkinson's and Alzheimer's diseases where the role of environmental and occupational toxic insults is conceivable[37].

Vitamin C (ascorbic acid) is an essential nutrient for humans, and is present within the brain due to regulated active transport[38]. The level of vitamin C in the brain is little influenced by external supply in normal state but if it is consumed in the brain increasingly due to an oxidative challenge, an extra amount given orally may support antioxidant defence. The lipid protecting effect of vitamin C *in vivo* has been demonstrated in iron-loaded guinea pigs[39]. Diminution of TBARS level by vitamin C in rats exposed to As via the drinking water was observed[40] – this is at variance with our result but in that work the dose of As was ca. 5 times lower. The cell protective effect of vitamin C against As was also demonstrated in human cancer-derived cells *in vitro*[41]. Vitamin C is a one-electron donor in redox reactions and can directly neutralize reactive oxygen species such as hydrogen peroxide, superoxide anion or the hydroxyl radical. It also regenerates alpha-tocopherol from its radical state[14].

Under certain circumstances, however, ascorbic acid can have a pro-oxidant effect[42], most typically if suitable transition metals (such as Fe) are present. Arsenic in known to release Fe^{3+} from ferritin[12], found, among others, in the RBCs; and the simultaneous presence of free iron and ascorbic acid gives way to reactions generating reactive oxygen species (such as the Fenton reaction). This phenomenon has been observed primarily *in vitro*[41] but may explain the increased level of TBARS and peroxynitrite in the RBCs of the animals in group *AsC* compared to those in group *As*.

The ability vitamin C to form a complex with As, yet another possible mode of action against the toxicity of As, has also been described[13].

Flavonoids are among the major groups of phytochemicals which are known to protect the organism against oxidative stress, and their potential in prevention and therapy has been intensively investigated. Their antioxidant effect can be considerable compared to that of ascorbic acid[43].

Green tea (the leaves and the drink - infusion - made of them alike) is a rich source of flavonoids, the main component being (–)-epigallocatechin-3-gallate. There is an increasing body of evidence that tea flavonoids can be protective against oxidative stress-dependent central nervous alterations including those caused by As[12,13]. In rats, signs of general toxicity and neurotoxicity induced by lead acetate were reduced by green tea in a dosage identical to ours[18]. Our results showed that green tea had apparently stronger protective effect than vitamin C against the neuro-functional damage caused by As, even if its effective dose, calculated in antioxidant capacity, was lower than that of vitamin C. This may result form the more complex actions of flavonoids. They act not only as direct radical scavengers but reduce oxidative stress and its consequences also by increasing the activity of the protective enzymes superoxide dismutase and catalase, and of anti-inflammatory pathways[16]. Further, the known metal chelating ability of tea flavonoids may have a role, acting either directly on As, or on Fe released from ferritin by methylated As[13]. Uptake of the flavonoids to human plasma[44] and to the brain of animals is promoted by the somewhat lipophilic character of the molecules[16] providing easier (and, theoretically, unlimited) access to the CNS while vitamin C is brought to the brain by regulated active transport[38].

The neuro-functional alterations caused by arsenic were efficiently diminished by two natural antioxidants in the present work. Antioxidants in food and drink –natural and easily accessible substances with advantageous biological effects – could thus be utilized for better protection against neurotoxic (and other) effects of arsenic, and also other environmental

toxicants, first of all in chronic low-level exposure such as that seen in South-East Hungary, provided that the effects suggested by the present results are verified in further experiments.

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Table 1

Treatment groups and doses

Treatment groups	**Codes**	**Dose and administration**	
		By gavage	Via drinking fluid
Control	*Con*	Distilled water (2 mL/kg b.w.)	Plain tapwater
Arsenic	*As*	10 mg As /kg b.w. (8.67 mg/ml $NaAsO_2$ in distilled water, 2 mL/kg b.w.)	Plain tapwater
Vitamin C	*VitC*	Distilled water (2 mL/kg b.w.)	Vitamin C, 1 mg/ml, dissolved in tapwater*
Arsenic + vitamin C	*AsC*	10 mg As /kg b.w. (8.67 mg/ml $NaAsO_2$ in distilled water, 2 mL/kg b.w.)	Vitamin C, 1 mg/ml, dissolved in tapwater*
Green tea infusion	*Tea*	Distilled water (2 mL/kg b.w.)	2.5 g green tea leaves brewed in 500 ml boiled water for 10 minutes
Arsenic + green tea infusion	*AsT*	10 mg As /kg b.w. (8.67 mg/ml $NaAsO_2$ in distilled water, 2 mL/kg b.w.)	2.5 g green tea leaves brewed in 500 ml boiled water for 10 minutes

* For dissolving vitamin C, tapwater was boiled short and cooled to room temperature to eliminate dissolved chlorine and oxygen. pH was set to 7.5 by adding $NaHCO_3$ to diminish breakdown of vitamin C (as suggested in[20].

Table 2

Summed As amounts received by the rats and tissue As levels

		Groups			
		Con	*As*	*AsC*	*AsT*
Summed external As dose, mg/rat		---	89.0±6.2	88.2±5.4	97.3±19.2
Tissue As (mg/kg dry weight)	RBCs	194.83± 11.043	1325.06± 44.22***	1338.41± 72.56***	1299.22± 268.11**
	Cortex	3.92±3.20	29.14± 11.88*	24.22± 4.81*	26.01± 5.99*
	Liver	4.94±3.02	19.84±13.03	23.10±7.59*	12.38±7.29
	Kidneys	10.09±7.64	396.61± 98.45*	467.49± 38.24**	432.97± 32.43**

Mean±SD, n=10 (external As dose) or n=3 (tissue As levels).

*, **, ***: $p<0.05$, 0.01, 0.001 vs. *Con.*

Table 3

Body weight gain of the rat groups during the 6 weeks of treatment

Groups	Body weight gain (g)
Con	232.85±25.11
As	184.31±23.43***
VitC	235.25±28.93
AsC	178.81±19.55***###
Tea	213.48±33.45
AsT	169.92±31.86***###

Mean±SD, n=10.

*, **, ***: p<0.05, 0.01, 0.001 vs. *Con*; #, ##, ###: p<0.05, 0.01, 0.001 vs. *VitC* or *Tea.*

Table 4

Summed antioxidant doses and oxidative stress indicators

		Groups			
		Con	*As*	*AsC*	*AsT*
Summed dose of antioxidant, in ascorbic acid equivalents, mg/rat[a]		---	---	142.65±23.77	63.47±20.53
H_2O_2 (μmol/mg protein)	RBCs	1.780±0.890	2.057±0.558	1.564±0.591	1.287±0.241[#]
	Cortex	0.595±0.027	0.656±0.052	0.543±0.065	0.665±0.060
ONO_2^- (nmol/mg protein)	RBCs	1.645±0.083	2.122±0.287*	2.434±0.565*	1.756±0.354
	Cortex	1.937±0.136	2.231±0.225	2.231±0.202	2.141±0.088
TBARS (nmol/mg protein)	RBCs	0.219±0.035	0.298±0.063*	0.318±0.047*	0.230±0.010
	Cortex	0.315±0.017	0.350±0.006*	0.360±0.050	0.300±0.015[#]

Mean±SD, n=3.

*: $p<0.05$ vs. *Con;* [#:] $p<0.05$ vs. *As*

[a]: The mean of summed antioxidant amounts was calculated for whole groups (n=10), based on the daily fluid consumption data and measured antioxidant activity of the corresponding drinking fluids. For the group *VitC*, it was 149.17±24.86 mg/rat.

Legends

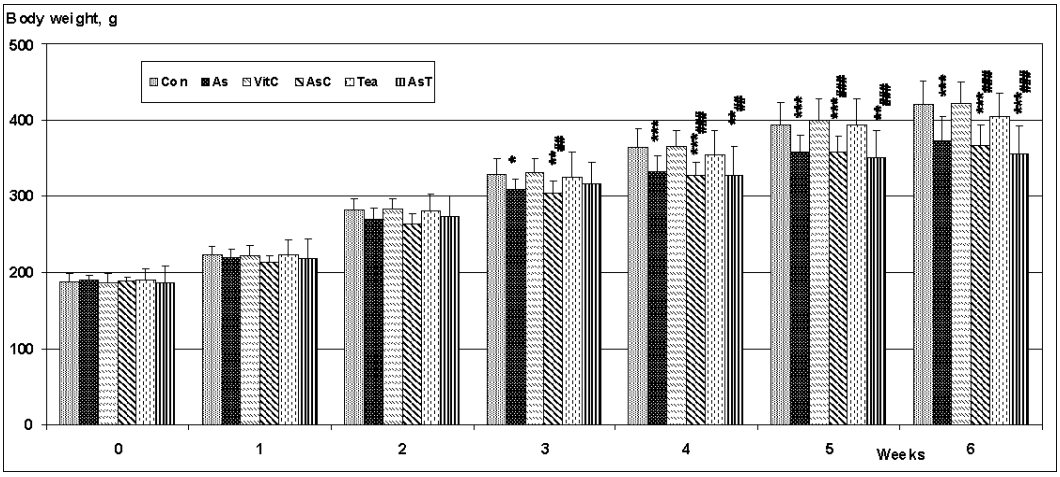

Fig.1

Time course of the rats' body weight gain in the control and treated groups. Mean+SD, n=10.

*, **, ***: p<0.05, 0.01, 0.001 vs. *Con*; $^{\#}$, $^{\#\#}$, $^{\#\#\#}$: p<0.05, 0.01, 0.001 vs. *VitC* or *Tea.*

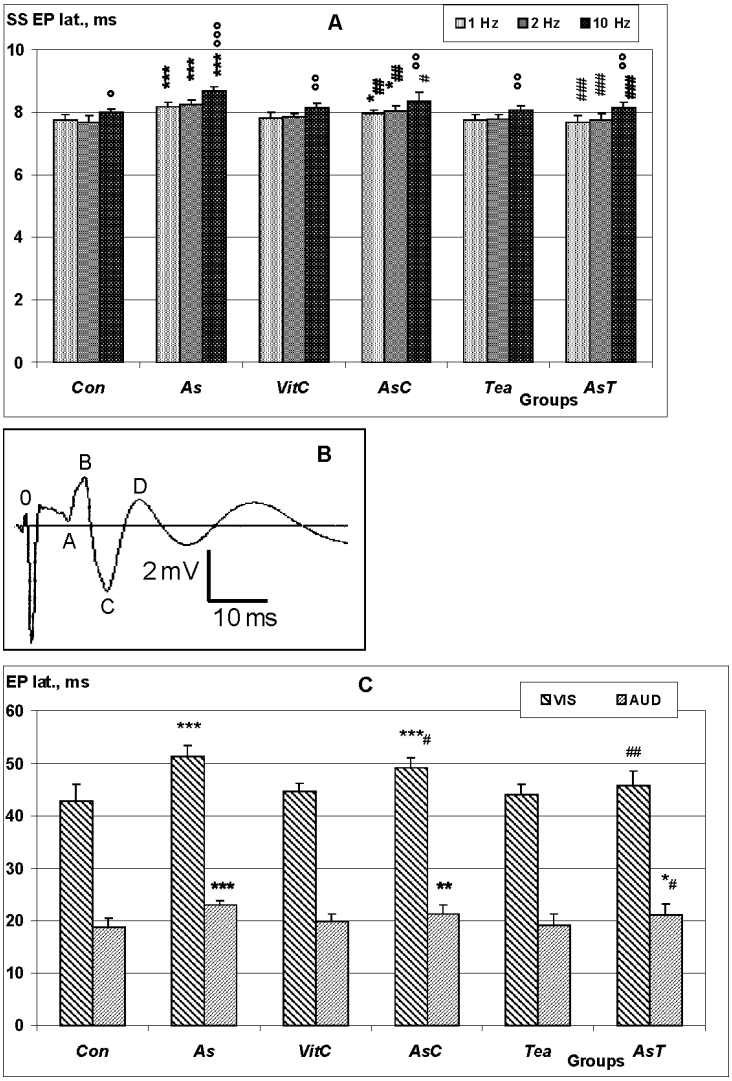

Fig 2

Latency of the cortical evoked potentials in the control and treated groups.

A, somatosensory EP latency (at various stimulation frequencies, see insert); B, measurement points on a sample somatosensory EP (see Materials and Methods); C, visual and auditory EP latency. Mean+SD, n=10.

*, **, ***: p<0.05, 0.01, 0.001 vs. *Con*; $^{\#}$, $^{\#\#}$, $^{\#\#\#}$: p<0.05, 0.01, 0.001 vs. *As*;

°, °°, °°°: p<0.05, 0.01, 0.001 vs. 1 Hz stimulation in the same treatment group.

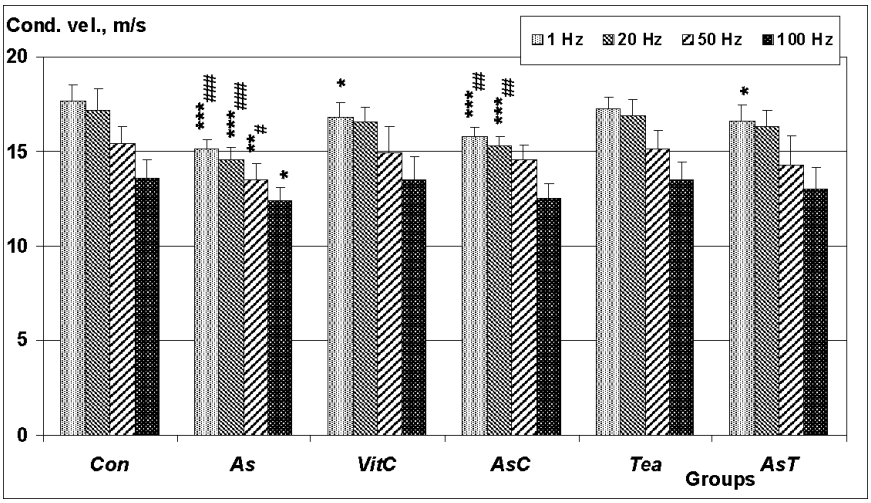

Fig 3

Conduction velocity of the tail nerve in the control and treated rats (at various stimulation frequencies, see insert). Mean+SD, n=10.

*, **, ***: p<0.05, 0.01, 0.001 vs. *Con*; $^{\#}$, $^{\#\#}$, $^{\#\#\#}$: p<0.05, 0.01, 0.001 *As* and *AsC* vs. *VitC*.